NEOGEN
REVEAL Q+ FOR DON
USING ACCUSCAN III READER

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GENERAL INFORMATION

The Reveal Q+ for DON test method provided by the Neogen Corporation is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of DON, using water as an extraction solvent along with a DON-antibody particle complex coated test strip and the Neogen AccuScan III reader.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	*Neogen Corporation 800/234-5333*
Test Kit Name:	Reveal Q+ for DON
Product Number:	8385
Effective Date of Instructions:	06/08/2015
Instructions Revision Number	0
Conformance Range:	0.5 – 5 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Wheat, corn, barley, malted barley, dried distillers grains with solubles (DDGS), oats, rice, corn gluten meal and wheat middlings
Extraction method:	Shake 50 grams sample in 250 mL of deionized water for 3 minutes.
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan III Reader only

PREPARATION OF TESTING MATERIALS

AccuScan III Reader Set-up:

(1) Launch the AccuScan III program on the reader.

(2) Select the Mycotoxin Q+ category and Q+ DON test.

(3) Enter a sample ID (optional).

(4) Enter the test's lot number, and A, B, C, and D values from the kit box or select from the drop-down menu next to History for previously entered lot number details.

Note: Technicians must verify/update test kit lot number in the AccuScan III reader program matches the lot number of the test strips in use before testing.

(5) Reading Test Results with the AccuScan III Reader.

Test strips should be read within 1 minute after completion of the 3 minute incubation period. Refer to Reveal AccuScan III manual for detailed set-up and selection information.

a. Fully insert the Reveal Q+ test strip into the black cartridge adapter with the sample end first and results facing out.

b. Insert the cartridge with test strip upside-down into the reader (the test lines will face downward into the reader).

c. The reader's green light will glow when a cartridge is inserted, and will automatically begin analyzing the cartridge.

d. The AccuScan III reader will analyze the test strip. Test results will be displayed and stored in the reader.

Note: Refer to the AccuScan III manual for more detailed instructions.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

a. Sample Preparation for wheat, corn, barley, corn gluten meal (CGM), distillers dried grains with solubles (DDGs), malted barley, oats, rice, and wheat middlings

The sample to be tested should be collected according to accepted sampling technique.

(1) Obtain a representative sample

(2) Grind the sample so that at least 95% of the ground material passes through a 20 mesh sieve, about the particle size of fine instant coffee.

b. Extraction Procedure for wheat, corn, barley, corn gluten meal (CGM), distillers dried grains with solubles (DDGs), malted barley, oats, rice, and wheat middlings

(1) Weigh 50 ± 0.2 grams ground samples into a blender cup.

(2) Add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.

(3) Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes. Allow the sample to settle for 3 minutes.

(4) Filter about 3 mL of sample extract using a Neogen syringe filter and collect the filtrate.

(5) Dilute the filtrate by two fold with distilled or deionized water. For example, add 1 mL of filtrate to 1 mL of distilled or deionized water. This is the diluted filter extract and ready for the analysis.

TEST PROCEDURES

a. Analysis Procedure

(1) Place the appropriate number of red sample dilution cups and clear sample cups for each test sample in the sample cup rack. Label cups if necessary.

(2) Using a single-channel pipettor with a new pipette tip, add 1000 microliters (µL) of sample diluent to each red sample dilution cup.

(3) Using a new pipette tip, add 100 µL of the diluted filter extract into each red dilution cup with sample diluents. Mix by swirling with the pipette tip and then by pipetting up and down 5 times, and transfer 100 µL into a new clear sample cup.

(4) Place a new Reveal Q+ for DON test strip with the sample end down into the sample cup. Start timer and incubate for 3 minutes.

(5) At the end of the 3 minute incubation/development period, remove the test strip from the sample cup. Read the test strip within one minute using only Neogen's AccuScan III Reader.

b. Reading the Results

(1) The strips must be immediately read using Neogen's AccuScan III Reader to analyze test strip. Test results will be displayed and stored in the reader.

(2) Fully inserted the Reveal Q+ test strip into the cartridge adapter with the sample end first and results facing out.

(3) Insert the cartridge with test strip side up into the AccuScan III.

(4) The reader will automatically begin analyzing the cartridge.

(5) The AccuScan III reader will analyze the test strip and test results will be displayed and stored in the reader.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (wheat only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation. The range for performance evaluation of quantitative DON test kits is 0.5 – 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 0.5 – 5.0 ppm for DON), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

Supplemental Dilution Procedure

(a) Combine 100 µL diluted filtered extract with 100 µL distilled or deionized water.

(b) Mix by swirling or pipetting up and down 5 times. This is the diluted filtered extract used for supplemental analysis.

(c) Refer to "**Test Procedures"** section for analysis.

(d) Read and record results on the work record, then multiply the analytical results obtained from the AccuScan reader by 2 to obtain the actual DON concentration of the original test sample (show all results on the work record).

Example: If the diluted filtered extract is diluted 1:1 (v/v) using extractions solvent, the dilution factor is two **(2)**.

AccuScan III reader supplemental analysis results:	4.0 ppm
Multiplied by the dilution factor	x 2
Sample results -**TOTAL:**	**8.0 ppm**

A final result less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5.0 ppm.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

Store kit components at room temperature (18-30ºC, 64-86ºF) to ensure full shelf life. Test strips should remain capped in their original tubes until used to ensure optimal performance.

b. Precautions

(1) Do not use test kit components beyond the expiration date.

(2) Ensure the device lot number and the curve details match the lot ID number selected on the reader. Failure to update the lot-specific A, B, C and D values on the AccuScan III reader will cause inaccurate results.

(3) The test strips must remain inside the stay-dry tube before use.

(4) Store test kit at room temperature 18-30°C, 64-86°F) when not in use, do not freeze.

(5) Treat all used liquids, including sample extract, and lab ware as if contaminated with DON. Gloves and other protective apparel should be worn at all times.

(6) To avoid cross-contamination, use clean glassware for each sample and thoroughly wash all glassware between samples.

EQUIPMENT AND SUPPLIES

a. <u>Materials provided in test kits.</u>

(1) 25 Reveal Q+ for DON test strips; 25 red sample dilution cups

(2) 25 clear sample cups; 2 bottles of sample diluent

(3) Instructions for use

b. <u>Materials required but not provided.</u>

(1) Timer (Neogen item #9426); 100 µL pipettor (Neogen item #9272, #9278)

(2) 100 µL pipette tips (Neogen item #9407, #9410, #9417); 500 µL pipettor (Neogen item #9291, #9336)

(3) 200-1000 µL pipette tips (Neogen item #9464, #9487, #9292, #9293); Sample collection cups with lids. (Neogen item #9428); Reveal sample rack. (Neogen item #9475)

(4) Reveal AccuScan III Reader (Neogen item #9595)

(5) Disposable polyethylene transfer pipettes; Dispensing pump or graduated cylinder (Neogen item #9448, #9447)

(6) Agri-Grind grinder or equivalent (Neogen item #9427); Scale capable of weighing 5 – 50 grams (Neogen item #9427)

(7) Bottle, 1 Liter (Neogen item #9472)

REVISION HISTORY

Revision 0 (06/08/2015)